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The use of the M-Vac[®] wet-vacuum system as a method for DNA recovery

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Abstract

Collecting sufficient template DNA from a crime scene sample is often challenging, especially with low quantity samples such as touch DNA (tDNA). Traditional DNA collection methods such as double swabbing have limitations, in particular when used on certain substrates which can be found at crime scenes, thus a better collection method is advantageous. Here, the effectiveness of the M-Vac[®] Wet-Vacuum System is evaluated as a method for DNA recovery on tiles and bricks. It was found that the M-Vac[®] recovered 75% more DNA than double swabbing on bricks. However, double swabbing collected significantly more DNA than the M-Vac[®] on tiles. Additionally, it was found that cell-free DNA is lost in the filtration step of M-Vac[®] collection. In terms of peak height and number of true alleles detected, no significant difference was found between the DNA profiles obtained through M-Vac[®] collection versus double swabbing of tDNA depositions from 12 volunteers on bricks. The results demonstrate that the M-Vac[®] has potential for DNA collection from porous surfaces such as bricks, but that alterations to the filter apparatus would be beneficial to increase the amount of genetic material collected for subsequent DNA profiling. These results are anticipated to be a starting point to validate the M-Vac[®] as a DNA collection device, providing an alternative method when DNA is present on a difficult substrate, or if traditional DNA collection methods have failed.

Keywords Touch DNA (tDNA), M-Vac, Wet-Vacuum, Double Swab, DNA profiling.

1. Introduction

DNA evidence plays a fundamental role in criminal investigations and the field of forensic science. In 2014/2015, the chance that a crime scene profile would match a profile previously stored in the UK National DNA Database was 63.2%, demonstrating the powerful effect that DNA evidence can have on the investigation of crimes [1]. Since the physical collection of DNA from a crime scene or object is the first step in the forensic DNA profiling process, it can be crucial to later obtaining an interpretable DNA profile.

Traditional and still widely used collection techniques, including taping [2], swabbing [3], and cutting [4], have certain limitations. Efficacy of these techniques is highly dependent on the substrate on which the DNA is deposited and the source of DNA [5]. DNA is commonly obtained from biological samples, including blood, semen, saliva, hair, and touch depositions resulting from skin contact. The latter, referred to as touch DNA (tDNA), is composed of both cellular and cell-free DNA and may result in part from sloughed off epithelial cells often carried by sweat and oil secretions [6,7]. Touch DNA can provide essential information in many types of forensic cases, allowing scientists to obtain a DNA profile from touched items including, but not limited to, firearms, drug packages, points of entry, tools, weapons, and clothing [8]. tDNA has proven to be very important in cases where other forms of biological evidence are absent [9].

The properties of tDNA compared to other biological samples pose challenges to scientists' ability to obtain DNA profiles. The primary issues with tDNA are that it is often present in very low quantities, and not visible to the naked eye, making collection difficult. Schultz & Reichert [10] report that the DNA quantity deposited from one fingerprint ranges from <0.01ng to 0.3ng. However, the amount of tDNA deposited is highly variable between individuals, and within the same individual at different times [11,6]. Since the optimal amount of DNA for most PCR kits is 1ng, scientists are often not able to acquire DNA profiles from touch samples due to insufficient template DNA. Additionally, since the aforementioned traditional collection techniques can generally only cover small areas, the amount of surface from which DNA can be collected is limited [5].

Another layer of difficulty is added to acquiring tDNA when it is present on a 'difficult' substrate. Bricks are known to be a difficult substrate to collect DNA from [12,5]. It is known that bricks are frequently used in assaults, burglaries, and riots, and often the only DNA evidence from the perpetrator(s) is tDNA left on them. The porosity and coarse nature of bricks present challenges to traditional DNA collection techniques. Both double swabbing as well as taping are described as difficult to use on bricks, due to the swab fraying and the tape losing its adhesiveness [5].

A novel DNA collection technique has been launched in an attempt to increase DNA collected from porous surfaces, as well as larger surface areas. The Microbial Vacuum (M-Vac[®]) Wet-Vacuum System (CSI Equipment Ltd, UK) was initially developed for use in the food industry as a device to sample food for potential pathogens [13]. The M-Vac[®] has recently been adapted as a DNA collection tool, targeting large and porous surfaces [14]. It functions by spraying a sterile buffer onto a surface, while simultaneously vacuuming up the liquid and any cells or DNA present into a sterile container (Figure 1). The resulting solution is then filtered through a 0.45µm polyethersulfone (PES) vacuum filter, and the biological material is retained on the filter. The filter can then be processed for DNA profiling following standard procedures. To date, there has been a limited amount of research on the use of the M-Vac[®] system in forensic science [5,15,16]; however, recent research has demonstrated that utilizing the M-Vac[®] on porous and large surface areas can result in increased DNA collection [5,15]. It has been found that the M-Vac[®] is more effective than swabbing when collecting DNA from surfaces including denim, carpet, laminate wood, and glass; however, small sample sizes limit the significance of the results from the latter two surfaces [5,15]. It has also been demonstrated that when collecting DNA from less porous surfaces like skin and tile, the M-Vac[®] resulted in DNA quantities very similar to what was obtained from double swabbing [5,16]. The research up to this point has not focused on touch DNA collection from porous surfaces using the M-Vac[®]; therefore, this study aimed to clarify the contradicting results in previous studies, and further the current research by focusing on touch DNA collection from porous substrates.

-----Figure 1 about here-----

Fig. 1 Schematic of the M-Vac[®] system. The sterile solution (1) is pressurised and projected through the tubing (2) to the sampling head. The solution is sprayed onto the surface and immediately vacuumed up, bringing with it any DNA and/or cellular material (3). The resulting solution is transferred to the collection bottle (4) as a result of the vacuum pressure exerted by the support equipment casing (5)

The system itself is straightforward to operate with a small amount of training needed. The user interface consists of a pressure gauge, a flow gauge, and three switches: power, solution pressure, and vacuum. Once the tubing is connected and the solution pressure has reached an appropriate level, the system is ready for use. Care must be taken when attaching the collection head as sterility must be maintained by avoiding external contact with the connection points, as these points come into contact with the collected sample. Following collection, the M-Vac[®] process requires additional time due to the filtration step. Although the collection process using the M-Vac[®] is more complex and time consuming than the double swabbing technique, the system is relatively straightforward, requiring limited training. It would be feasible to train police and crime scene officers to use the M-Vac[®] at a crime scene, increasing its potential value as a tool in forensic science.

The aim of the present study was to determine the feasibility of the M-Vac[®] as a DNA collection device, using human saliva and touch samples as sources of DNA. The filter apparatus supplied with the M-Vac[®] was investigated to determine if DNA is lost in the filtration step of the M-Vac[®] collection process. The DNA recovery using the double swabbing method was compared to the DNA recovery using the M-Vac[®] on both tiles and bricks. Finally, DNA profiling was done to evaluate results

from touch samples on bricks, and profile quality was compared between the samples from the double swabbing and M-Vac® techniques.

2. Materials and Methods

2.1 Determining DNA retention in the filter

A GeneRuler 50 base pair DNA Ladder (Thermo Fisher Scientific), ranging in fragment sizes from 50-1000 base pairs was diluted in 15mL sterile distilled water (SDW) to provide sufficient liquid, and put through the vacuum filter connected to the M-Vac® support equipment casing (SEC) (n=3). The filter was removed and pulse vortexed with 15mL of water to allow any cellular material or DNA present to loosen and release from the filter. Both the filter solution (F), containing any DNA trapped on the filter, and the filtrate (FT), containing any DNA that was not retained on the filter, were concentrated in Amicon Ultra-15 10K sample reservoirs (Merck Millipore, Ireland) and centrifuged at 4,000 RPM for 1 hour. The resulting concentrates were run alongside the DNA ladder and a negative control on a 2.5% agarose gel at 50V for 1 hour, and then visualised.

2.2 Saliva collection and analysis

All the experiments were performed in compliance with the UK law and the institutional guidelines. Ethical approval for sample collection was granted by the Institution Research Ethics Committee (BDM/12/13-101). Informed consent was obtained for all sample collections.

Saliva (10mL) was collected in a tube from a consenting donor and 50µL of the saliva sample were then extracted in triplicate using the QIAgen Micro™ kit (QIAgen®, U.K.) following the “Small Volumes of Blood” protocol. All extractions included carrier RNA and were eluted in 20µL of SDW. The concentration of DNA in the extracted saliva sample was determined using the Quantifiler™ Human DNA Quantification kit (Applied Biosystems) by performing triplicate quantifications of each extract. The quantification reactions were performed on an ABI Prism 7500 Fast Real-Time PCR System and SDS software version 2.3 (Applied Biosystems).

The saliva sample was diluted to a sample containing approximately 2ng of DNA per 50µL, creating a more realistic concentration of tDNA. The resulting diluted solution was extracted in triplicate and quantified in triplicate, as described above.

2.3 DNA recovery from tiles

2.3.1. Deposition of DNA and collection from tiles

50µL of the diluted saliva solution were then spiked onto tiles that were UV irradiated prior to use. 50µL were spiked in the centre of one half, and another 50µL in the centre of the other half. The tiles were allowed to dry overnight. A negative substrate control was performed to confirm the absence of any DNA.

The saliva was collected using the double swabbing technique on one side of the tile, and the M-Vac® on the other side (n=5). The double swabbing procedure consisted of a cotton swab being moistened with SDW, and the entire surface then swabbed using a side-to-side motion. The same surface was then swabbed with another dry swab to collect any residual liquid and optimise DNA recovery. The two swabs for each sample were combined for processing. Collection using the double swabbing technique was performed first to avoid cross contamination, due to the propensity of the M-Vac® system to propel cellular material during collection due to the pressurized spraying of the sterile buffer [5]. The M-Vac® collection was carried out with a new sampling head for each sample. Sampling consisted of a forward and backward dragging motion, as per manufacturer's recommendations. The sampling head was run over the entire surface of the tile once with the solution spray on, and once with the solution spray off to collect residual liquid remaining on the tile. The sampling volume was standardized at ~50mL of solution collected per sample. A schematic of the mechanism of the M-Vac® is provided in Figure 1. The resulting solution collected by the M-Vac® was then poured through the Nalgene™ Rapid-Flow™ 0.45µm Filter with a PES Membrane (Thermo Fisher Scientific), connected to the M-Vac® SEC. The filter units were allowed to dry overnight prior to extraction. Appropriate steps were undertaken to avoid contamination, including wearing of gloves, mask, and scene suit, and all sample collection being performed in a laminar flow hood.

2.3.2. Extraction and quantification of collected samples from tiles

All samples were extracted as described in 2.2, using the “Isolation of Total DNA from Surface and Buccal Swabs” protocol, including carrier RNA, and eluted in 20µL. For all M-Vac® samples, a sterile scalpel was used to cut the dried filter into strips roughly 5mm wide, and then transferred into a 2mL Eppendorf tube. The filter samples were then extracted following the same procedure as the double swab samples. The extracts were quantified as described above.

2.4 DNA recovery from bricks

New bricks were purchased and in order to estimate an average background level of DNA on the bricks using each collection method, bricks were sampled using both the M-Vac[®] and double swabbing prior to the spiking of any DNA (n=6). Half of each brick was sampled using the double swabbing technique, and the other half using the M-Vac[®] following the same technique as described above. On new bricks, 50µL of neat saliva were spiked onto the centre of one half of each brick, and 50µL onto the centre of the other half (n=15). Neat saliva was chosen as opposed to a diluted solution of saliva due to the poor recovery rate of DNA from bricks found in previous studies [5]. The bricks were allowed to dry overnight. The collection procedure was as described in 2.3.2, with the double swabbing performed first followed by the M-Vac[®] collection. Extraction and quantification followed the same procedure as described in 2.2.

2.5 Touch DNA samples recovered from bricks

2.5.1. Touch DNA deposition

Twelve volunteers were instructed to not wash their hands for at least an hour prior to tDNA deposition to mimic a real scenario. A 30 second 'grooming period' followed in which volunteers were asked to rub their hands on their neck and face, followed by rubbing their hands together in an attempt to equalize the amount of DNA on each hand [17]. Each volunteer then placed their hands equal distances from a line drawn through the centre of the top surface of one brick, ensuring that both palms were in equal contact with the brick. Volunteers applied the same pressure to each hand for 30 seconds, and then slid their hands off the brick towards them, mimicking the hand contact pattern that would occur had the brick been thrown.

2.5.2. DNA profiling of touch samples

Sample collection, extraction, and quantification were carried out as described previously. STR analysis was performed using the AmpFLSTR[®] NGM Select[™] PCR Amplification Kit (Applied Biosystems) using a 25µL reaction volume. Amplification was performed on a GeneAmp[®] PCR System 9700 (Applied Biosystems) using 29 cycles as per manufacturer's recommendations. Amplified products were then profiled using capillary electrophoresis on a 3130XL Genetic Analyser (Applied Biosystems). The limit of detection for the designation of alleles was 50 RFU.

3. Results and Discussion

3.1 DNA retention in the filter

Compared to double swabbing, the M-Vac[®] collection process includes an additional step, pouring the collected solution through a filter to concentrate the DNA. Therefore, it was important to determine if DNA is lost during this step. One previous study has looked at the filter supplied with the M-Vac[®] to determine if any DNA loss occurs at the filtration step, and concluded that no DNA is lost [18]. However, the results from [18] are not applicable to cell-free DNA since the filtrate was tested for DNA utilising a Chelex extraction method, which would remove any cell-free DNA present in the sample [19]. As a result, the conclusion in [18] that no DNA is detected in the filtrate only applies to cellular DNA, and not cell-free DNA, since cell-free DNA would have been discarded at the extraction step.

The aim of this experiment was to determine if the smaller, cell-free fragments of DNA are retained during M-Vac[®] processing. A DNA ladder was used as a source of cell-free DNA in fragments around 60-100 base pairs, because these fragment lengths have been demonstrated to be present in healthy individuals [20]. The results show that all DNA fragments were detected in the FT samples, and no DNA was detected in the F samples, demonstrating that cell-free DNA is not retained on the filter during the filtration step.

These results were expected due to the fact that the filter supplied with the M-Vac[®] is made of PES, which does not chemically interact with the DNA molecule, but filters by size alone. The 0.45µm filter would be expected to trap cells and some cellular debris, but DNA, either a ladder as in this experiment or endogenous cell-free DNA molecules as reported in humans, is smaller and would be expected to pass through. This clearly demonstrates that cell-free DNA is not retained on the filter, and thus would not be included in subsequent DNA profiling.

The loss of cell-free DNA represents a drawback to the M-Vac[®], since cell-free DNA has been found to contribute significantly to recoverable amounts of DNA in sweat and touch samples, as well as increase the detected alleles in touch DNA profiles, providing greater statistical weight to potential matches with a National DNA Database or suspect reference sample [19]. An additional drawback of the M-Vac[®] technology is the large volume of filtrate obtained during the filtration step of M-Vac[®] samples (dependent on the surface area of the sampled object), which prevents the filtrate from being extracted directly without the use of a concentration method prior to extraction, thus introducing another stage for potential loss. The volume of solution that can be collected in one collection bottle can exceed 200mL. Since the double swabbing technique does not require the additional filtration step, cell-free DNA is collected and proceeds to further processing. If the filtration step is altered in the M-Vac[®] process, and a concentration method that will retain cell-free DNA is utilised, it is hypothesised that the M-Vac[®] will likely result in an even higher DNA yield than demonstrated in this study.

In addition to DNA loss due to filtration or concentration, the sterile buffer could also represent a drawback of the M-Vac[®] collection. The M-Vac[®] has been marketed as a portable system that can be taken to crime scenes to sample objects. Following collection, the collection bottle containing the sterile solution and the potential DNA can be closed and sent to the laboratory for processing. The sterile solution, sold as Butterfield's Buffer, is composed of sterile distilled water with phosphate as a

buffer to stabilise the pH. Due to the differing salt concentrations between the buffer and human cells, osmotic movement could cause cells to lyse if left in the buffer for too long, causing DNA to be released from the cells.

Since the present study demonstrated that cell-free DNA is lost in the filtration step, the filtration should be performed promptly following collection, or it is hypothesised that DNA could be lost after filtering due to the cells lysing, rendering the sample unusable. However, filtration at a crime scene is also not ideal as processing in the laboratory is typically carried out by more trained personnel, and provides less opportunity for contamination. To solve this problem, the buffer currently used could potentially be replaced with phosphate buffered saline (PBS) as this would allow cells to stay intact, and does not inhibit subsequent DNA profiling [21].

3.2 Percent recovery of DNA from tiles and bricks

To test the feasibility of the M-Vac[®] system, the percent recovery of DNA was calculated to determine whether or not the M-Vac[®] collects more DNA than double swabbing. Saliva was chosen as the source of DNA to most closely mimic touch DNA due to its composition including both epithelial and cell-free DNA, as well as its invisibility to the naked eye [19]. Saliva composition is better understood and it is more predictably quantifiable than typical touch DNA samples.

Percent recovery from tiles was calculated based on the relative quantification values of the amount of DNA spiked onto each tile, and the amount of DNA recovered. A t-test was employed to compare the mean values and demonstrated that the double swabbing method on tiles yielded samples with significantly more DNA on average than the M-Vac[®] ($p < 0.05$), as shown in Figure 2.

-----Figure 2 about here-----

Fig. 2 Percent recovery of DNA on both bricks ($n=15$) and tiles ($n=5$). Collection was performed using either the double swabbing method or the M-Vac[®].

Consistent with previously published results from [5], it was found that the M-Vac[®] collected less DNA than double swabbing on tiles. Garret et al. reported that on tiles, the pressure of the solution spraying from the M-Vac[®] propels DNA up to 4 inches from the collection area, which could contribute to the loss [5]. During the current study it was observed that the nubs on the M-Vac[®] head, designed to prevent the sampling head from suctioning onto surfaces, prevented some of the expelled solution from being vacuumed back up into the collection bottle. As a result, the M-Vac[®] could be leaving DNA on the surface that the swabs were able to collect. It is conceivable that this issue is more prominent on non-porous surfaces like tiles than porous surfaces like bricks, since the pores on porous surfaces would prevent the swabs from collecting some DNA present.

Since the M-Vac[®] is marketed as a system for use on large and porous surface areas, it was of interest to test the percent recovery of DNA on bricks, a very porous surface. Percent recovery from bricks was calculated in the same manner as it was for tiles, after subtracting background DNA. Quantification values for background DNA ranged from 0 to 0.013ng/μL and 0 to 0.018ng/μL for double-swabbing and M-Vac[®] collection, respectively. No negative values were obtained when calculating percent recovery. When recovering DNA from bricks, the M-Vac[®] was shown to be significantly better than the double swabbing method ($p < 0.05$). The M-Vac[®] collected samples yielding significantly more DNA than the double swabbing method ($p < 0.05$), with an average of 75% more (Figure 2). It is possible that the M-Vac[®] is able to collect DNA from the pores of the brick whereas double swabbing is only able to collect from the surface, which would explain the results obtained. Although it was attempted to closely mimic tDNA, the setup of the experiment potentially deviates from real scenario conditions. It is hypothesised that the majority of DNA deposited from touch samples would be present on the surface of the brick. Since the saliva was pipetted onto the surface, it was absorbed into the pores due to it being a liquid. Although these results do not fully reflect the effectiveness of the M-Vac[®] collecting touch depositions, the results demonstrate the effectiveness of collecting DNA deposited in liquids, like body fluids. The results show the ability of the M-Vac[®] to collect more DNA, potentially by penetrating the pores of the brick, allowing a greater DNA yield than the traditional double swabbing collection.

3.3 DNA profiles from touched bricks

In order to determine whether or not a DNA profile could be obtained from the handling of bricks, 12 consenting volunteers were asked to deposit tDNA on bricks, as described in section 2.4. Samples were processed as described previously, with DNA extraction following the "Isolation of Total DNA from Surface and Buccal Swabs" protocol. Quantification values for the DNA collected from each sample ranged from 0.002 to 0.312ng/μL and 0 to 0.707ng/μL for double-swabbing and M-Vac[®] collection, respectively. No distinct trends were observed in the quantification values when comparing each collection method. However, the purpose of this experiment was to compare results at the DNA profile level, and quantification was performed only to ensure appropriate DNA quantities were carried on to the capillary electrophoresis. The profiles obtained from each volunteer using both collection methods were evaluated based on the number of true alleles and peak heights.

The results obtained offered no distinct trend regarding which collection method results in better quality DNA profiles. When comparing the number of true alleles, the M-Vac[®] collection resulted in a greater average number of true alleles; however, the results are not statistically significant ($p > 0.05$). Furthermore, peak heights were also very variable within individuals, and offered no conclusion as to which collection method is superior. When evaluating peak heights at each locus, seven out of the

twelve volunteers provided statistically significant differences comparing the peak heights of the M-Vac[®] sample to the double swab sample. A summary of the results is provided in Table 1.

Despite the significant quantification results obtained in 3.2, the superiority of the M-Vac[®] system was not reflected at the DNA profile level. The results demonstrate that it is possible to obtain DNA profiles from brick using both double swabbing and the M-Vac[®]; however, the results were varied and offer limited bases for conclusion as to which collection method is superior.

The varied results could be due to a number of factors. tDNA depositions have high inter- and intra-individual variability, potentially contributing to the inconsistent results, despite efforts to equalize the amount of DNA on each hand [22,6]. Additionally, profiles resulting from a low quantity of template DNA are often subject to stochastic effects, including peak height variation among replicates of the same sample, possibly contributing to the lack of conclusive results [23]. The results from 3.2 demonstrate that the surface on which DNA is deposited affects the collection of DNA, supporting the findings in [5]. Therefore, the variable results could also be a product of the variability of the brick surfaces. Despite the variability, the M-Vac[®] collection resulted in full profiles in 50% more cases than the double swabbing collection. The M-Vac[®] collection also resulted in, on average, a greater number of true alleles than double swabbing (25.4 versus 23.7 alleles respectively); however, the results are not statistically significant. The results obtained in this study demonstrate promising evidence of the capabilities of the M-Vac[®] to collect touch DNA from bricks, but the variability and lack of statistically significant results presents the need for further testing before the M-Vac[®] can be considered superior or inferior to double swabbing at the DNA profile level.

Table 1 Summary of the DNA profiles obtained from touch DNA on bricks. Half of each brick was sampled using double swabbing (DS) and the other half using the M-Vac[®] (M). The number of true alleles amplified are out of a possible 34

Volunteer	Collection Method with Greater Peak Heights	Number of True Alleles Amplified	
		Double Swabbing	M-Vac [®]
1	M**	33*	34*
2	DS**	34	30
3	DS**	34	34
4	M**	16*	34*
5	M	4*	31*
6	M**	33	34*
7	M	11*	8
8	DS	28*	8
9	M**	34*	34
10	DS	7	10
11	DS**	33	34*
12	M	17*	14*

*profile obtained was a mixed profile. Mixed profiles ranged from 1 to 3 alleles not belonging to the donor.

**demonstrated statistically significant differences ($p < 0.05$) between the DS sample and M-Vac[®] sample in peak heights at each allele, using a paired t-test.

5. Conclusions

The data presented in this study demonstrates that the M-Vac[®] has the potential to be a useful tool in the field of forensic science. While it was demonstrated that the M-Vac[®] collects more DNA from deposited saliva than double swabbing on bricks, more research must be done on different types of surfaces, as it is known that the amount of DNA collected is not only dependent on the collection method, but also the surface from which the DNA is being collected. Modifications should be made regarding the concentration method of the collected solution to allow cell-free DNA to be included in DNA profiling, and likely increase the quality of the resulting profiles. The buffer solution should also be changed to allow for storage of the collected sample prior to the filtration step without losing any additional DNA. In addition to the improvements that should be made to the M-Vac[®], the feasibility of use at a crime scene as well as the cost must be considered prior to adoption by forensics labs and police forces.

Despite the limitations and need for further research, the results obtained provide increasingly positive evidence that the M-Vac[®] can be a useful tool in the field of forensic science. It has been supported that the M-Vac[®] is superior at collecting DNA from saliva on bricks, a substrate that poses problems to traditional DNA techniques. If the suggested manipulations are implemented, the M-Vac[®] has the potential to be a very useful forensic tool in the future. At the present time the use of this system could be evaluated in those cases where traditional methods have failed.

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References

1. Home Office, National DNA database Strategy board Annual report 2014-15 (2015).
2. Verdon TJ, Mitchell RJ, van Oorschot RAH (2014) Evaluation of tapelifting as a collection method for touch DNA. *Forensic Sci Int-Gen* 8 (1):179-186. doi:10.1016/j.fsigen.2013.09.005
3. Pang BC, Cheung BK (2007) Double swab technique for collecting touched evidence. *Leg Med (Tokyo)* 9 (4):181-184. doi:10.1016/j.legalmed.2006.12.003
4. Petricevic SF, Bright JA, Cockerton SL (2006) DNA profiling of trace DNA recovered from bedding. *Forensic Sci Int* 159 (1):21-26. doi:10.1016/j.forsciint.2005.06.004
5. Garrett AD, Patlak DJ, Gunn LE, Brodeur AN, Grgicak CM (2014) Exploring the potential of a wet-vacuum collection system for DNA recovery. *Journal of Forensic Identification* 64 (5):429-448
6. Quinones I, Daniel B (2012) Cell free DNA as a component of forensic evidence recovered from touched surfaces. *Forensic Sci Int-Gen* 6 (1):26-30. doi:10.1016/j.fsigen.2011.01.004
7. Wickenheiser RA (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J Forensic Sci* 47 (3):442-450
8. Raymond JJ, van Oorschot RAH, Gunn PR, Walsh SJ, Roux C (2009) Trace DNA success rates relating to volume crime offences. *Forensic Sci Int-Gen* 2 (1):136-137. doi:<http://dx.doi.org/10.1016/j.fsigs.2009.08.072>
9. Williamson AL (2011) Touch DNA: forensic collection and application to investigations. *Journal of the Association for Crime Scene Reconstruction* 18 (1):1-6
10. Schulz MM, Reichert W (2002) Archived or directly swabbed latent fingerprints as a DNA source for STR typing. *Forensic Sci Int* 127 (1-2):128-130
11. Lowe A, Murray C, Whitaker J, Tully G, Gill P (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int* 129 (1):25-34
12. Avraham COS, Berlyne S, Gafny R, Hazan-Eitan Z, Cohen A (2015) Forensic DNA analysis from rocks and stones in criminal cases. *Journal of Forensic Identification* 65 (5):793-802
13. Ryan S, The M-Vac: a valuable new collection tool, <http://www.m-vac.com/news/news-posts/the-m-vac-a-valuable-new-collection-tool-by-suzanna-ryan>, (accessed 27 March 2017).
14. How does the M-Vac work?, <http://www.m-vac.com/why-mvac/how-it-works>, (accessed 27 March 2017).
15. Hedman J, Agren J, Ansell R (2015) Crime scene DNA sampling by wet-vacuum applying M-Vac. *Forensic Sci Int-Gen* 5:E89-E90. doi:10.1016/j.fsigs.2015.09.036
16. Williams S, Panacek E, Green W, Kanthaswamy S, Hopkins C, Calloway C (2015) Recovery of salivary DNA from the skin after showering. *Forensic Sci Med Pathol* 11 (1):29-34. doi:10.1007/s12024-014-9635-7
17. Sewell J, Quinones I, Ames C, Multaney B, Curtis S, Seeboruth H, Moore S, Daniel B (2008) Recovery of DNA and fingerprints from touched documents. *Forensic Sci Int-Gen* 2 (4):281-285. doi:10.1016/j.fsigen.2008.03.006
18. M-Vac Systems, Filter apparatus verification, 0.45 µm PES filter material including comparison of double swab method and M-Vac with saliva stained cotton.
19. Vandewoestyne M, Van Hoofstat D, Franssen A, Van Nieuwerburgh F, Deforce D (2013) Presence and potential of cell free DNA in different types of forensic samples. *Forensic Sci Int-Gen* 7 (2):316-320. doi:10.1016/j.fsigen.2012.12.005
20. Mouliere F, Robert B, Peyrotte EA, Del Rio M, Ychou M, Molina F, Gongora C, Thierry AR (2011) High fragmentation characterizes tumour-derived circulating DNA. *Plos One* 6 (9):1-10. doi:ARTN e23418
21. Martin NC, Pirie AA, Ford LV, Callaghan CL, McTurk K, Lucy D, Scrimger DG (2006) The use of phosphate buffered saline for the recovery of cells and spermatozoa from swabs. *Sci Justice* 46 (3):179-184. doi:10.1016/s1355-0306(06)71591-x
22. Phipps M, Petricevic S (2007) The tendency of individuals to transfer DNA to handled items. *Forensic Sci Int* 168 (2-3):162-168. doi:10.1016/j.forsciint.2006.07.010
23. Balding DJ (2013) Evaluation of mixed-source, low-template DNA profiles in forensic science. *P Natl Acad Sci USA* 110 (30):12241-12246. doi:10.1073/pnas.1219739110

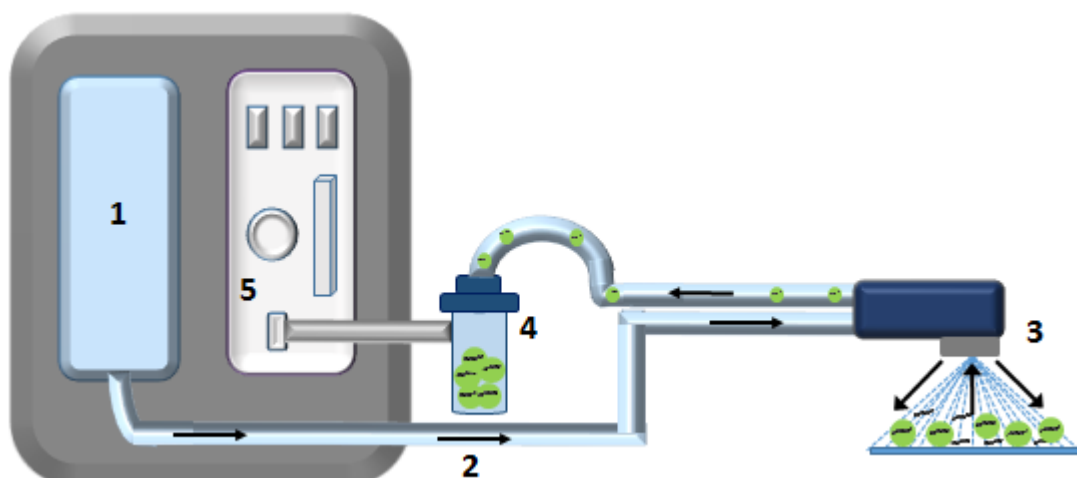


Fig. 1

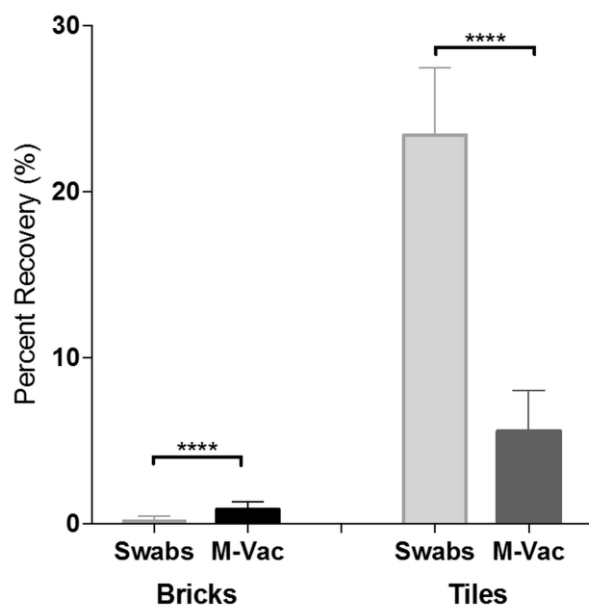


Fig. 2

Highlights

- The M-Vac[®] was superior to double swabbing for DNA collection from brick surfaces.
- Double swabbing collected more DNA than the M-Vac[®] on non-porous tiles.
- Cell-free DNA is lost in the filtration step of M-Vac[®] processing.
- The M-Vac[®] is a useful option for DNA collection when traditional methods fail.